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More Pieces of the Puzzle: New Insights into Azoxystrobin Exposures and Neurotoxicity

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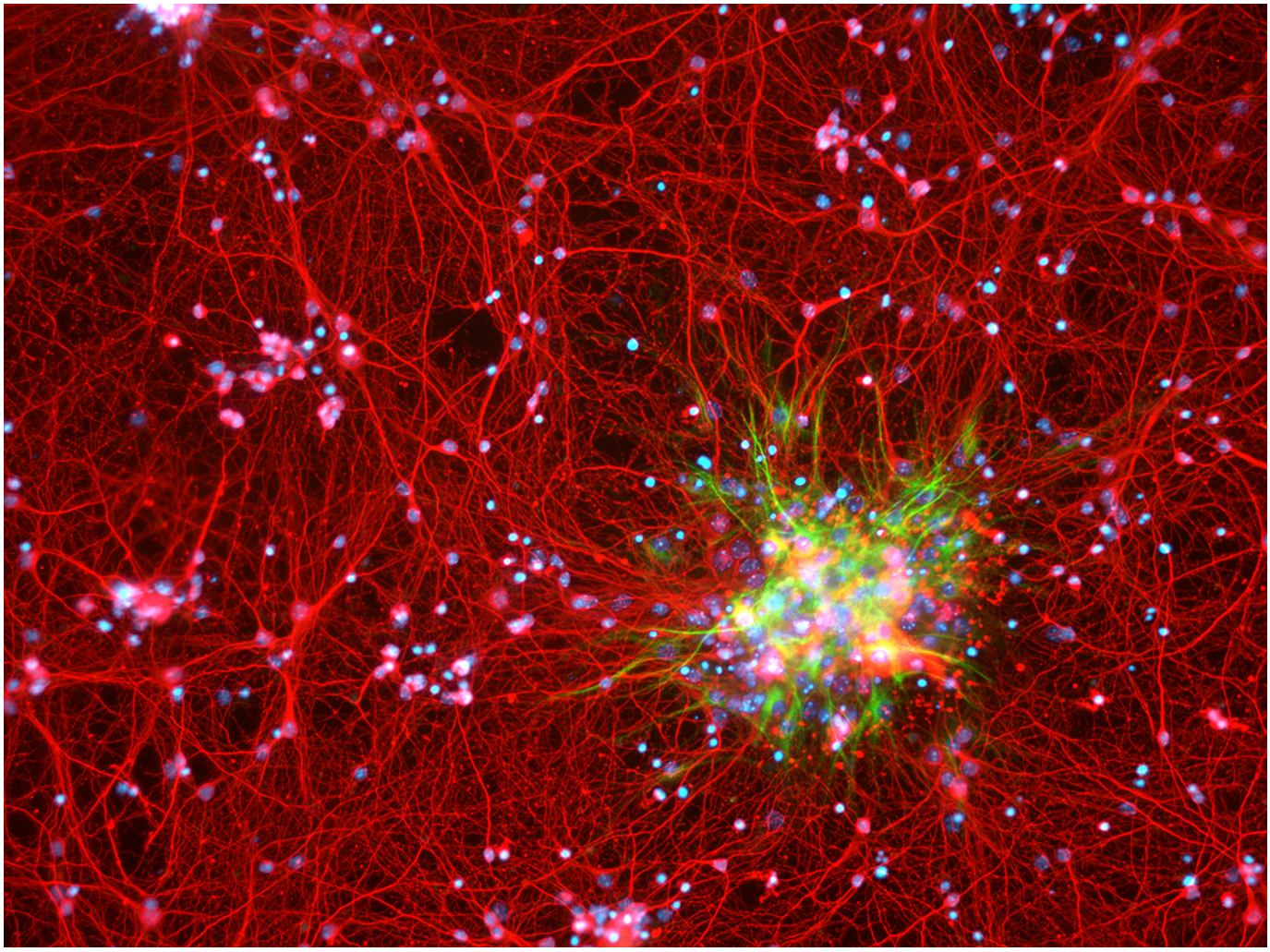
Azoxystrobin (AZ), a broad-spectrum fungicide, is seemingly everywhere. It is applied to a variety of crops, including cereals, potatoes, tomatoes, fruits, nuts, wine grapes, leafy greens, herbs, and spices.[1,2] Growers may also spray this versatile fungicide on turf grass, Christmas trees, animal feed, cotton, and ornamental plants.[3] AZ is even used in mold- and mildew-resistant wallboard.[4] And according to the findings of a new study in *Environmental Health Perspectives*, the chemical may well be in our bodies, too.[5]

Experimental evidence indicates AZ is toxic to the developing embryo,[6,7,8,9] but little is known about the extent of people's exposures, how they are exposed, or what health effects AZ may cause. Given its widespread use in agriculture, landscaping, and (since 2009) construction, AZ is increasingly viewed as a prime candidate for further study of human exposure and health impacts. In 2019, investigators with the National Institutes of Health's Environmental Influences on Child Health Outcomes (ECHO) program recommended AZ as one of 36 chemicals to prioritize for population-wide biomonitoring.[10] Another recent paper highlighted the chemical's relatively new use in wallboard and confirmed its presence in house dust.[4]

In the present study, researchers with the University of North Carolina (UNC) at Chapel Hill conducted experiments with human liver cells that identified a chemical called AZ-acid as a key metabolite of AZ. Experiments in mice further demonstrated that AZ-acid is a sensitive indicator of exposure to AZ. Finally, the researchers applied their findings to urine samples collected from 8 pregnant women and 67 children 3–7 years of age. The women provided samples between weeks 8 and 15 of gestation; four of the women provided an additional sample later in pregnancy. The



Cultured mouse cortical neurons (red) and astrocytes (green) at 10× magnification. Newly reported mouse experiments showed that azoxystrobin and its metabolite transferred from dams to offspring through the placenta and via lactation and that even relatively low exposures induced cortical cell death in embryos. Image: dchordpdx/CC-BY-4.0.

children's samples were collected at 3–7 years of age; 20 of the children provided an additional sample 9–12 months later.

AZ-acid was found in 100% of the samples from women and 70% of the samples from children. This suggests AZ exposure may be common in both adults and children, says Heather Stapleton, a professor at Duke University and senior author of the recent wallboard paper.[4] Moreover, says Stapleton, who was not involved in the new study, the authors identified a specific biomarker that can now be used to further evaluate exposure in a broader population: "This research . . . is an important next step to evaluate the primary sources of exposure and understand the health risks."

On the latter front, additional experiments in the new study showed that AZ and AZ-acid could transfer from pregnant mice to their fetal offspring and that pups could be exposed to the chemicals through milk. Because both chemicals were detected in the cerebral cortices of exposed animals, the researchers also assessed the toxicity of AZ and AZ-acid in embryonic mouse brain cells. They concluded that AZ promoted cell death and was highly neurotoxic at concentrations relevant to human exposures.

"The effects of these chemicals might be more of an issue for the developing brain," says senior author Mark Zylka, a UNC professor of cell biology and physiology who also served as coauthor of Stapleton's wallboard paper. "Some children had high levels in their urine, and in at least two of the children, we found that the levels were consistently high across two ages. That suggests there might be a persistent exposure to this chemical in some individuals."

Deborah Bennett, coauthor of the 2019 ECHO paper[10] and an exposure scientist at the University of California, Davis, says these findings reinforce the need to prioritize AZ for population-wide biomonitoring. "When you have the potential for both exposure and toxicity, then . . . we want to understand what the potential effects are for humans," says Bennett, who was not involved in the new work. "And the first step for that is being able to quantify exposure."

Although the current study's exposure assessment was limited to a small sample of subjects living in a single geographic area, the researchers' investigation of AZ-acid as a novel biomarker is of high quality, follows best practices, and provides "very compelling evidence of exposure," notes Elizabeth Marder, a senior environmental scientist with the California Environmental Protection Agency's Office of Environmental Health Hazard Assessment.

Marder, who was not involved in the new study, agrees that future research should assess AZ-acid in larger, more diverse populations. She also cites the need to evaluate temporal and spatial variability in exposure and investigate specific exposure sources, particularly in terms of diet vs. indoor environment. "What we're seeing here are pieces of the puzzle, but there's not yet enough to complete the puzzle," she explains. "We have some good edge pieces, but it's not a complete picture."

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